Characterizing leaf gas exchange responses of cotton to full and limited irrigation conditions

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ABSTRACT

Plant responses to water deficit need to be monitored for producing a profitable crop as water deficit is a major constraint on crop yield. The objective of this study was to evaluate physiological responses of cotton (*Gossipium hirsutum*) to various environmental conditions under limited water availability using commercially available varieties grown in South Texas. Soil moisture and variables of leaf gas exchange were measured to monitor water deficit for various varieties under different irrigation treatments. Lint yield and growth variables were also measured and correlations among growth parameters of interest were investigated. Significant differences were found in soil moisture, leaf net assimilation (A_n), stomatal conductance (g), transpiration rate (T_r), and instantaneous water use efficiency (WUE_i) among irrigation treatments in 2006 while no significant differences were found in these parameters in 2007. Some leaf gas exchange parameters, e.g., T_r, and leaf temperature (T_L) have strong correlations with A_n and g. A_n and WUE were increased by 30–35% and 30–40%, respectively, at 600 μmol (CO_2) m^{-2} s^{-1} in comparison with 400 μmol (CO_2) m^{-2} s^{-1}. Lint yield was strongly correlated with g, T_r, WUE, and soil moisture at 60 cm depth. Relative A_n, T_r, and T_L started to decrease from FTSW 0.3 at 60 cm and FTSW 0.2 at 40 cm. The results demonstrate that plant water status under limited irrigation management can be qualitatively monitored using the measures of soil moisture as well as leaf gas exchange, which in turn can be useful for describing yield reduction due to water deficit. We found that using normalized A_n, T_r, and T_L is feasible to quantify plant water deficit.

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1. Introduction

Crop growth and yield are influenced by plant genetic factors as well as environmental factors such as weather conditions, water availability, and soil conditions. Plant water is one of the most important and readily manageable variables for producing a profitable crop (Kozlowski, 1972; Taylor et al., 1983). Stresses involving water deficiencies will adversely affect cell turgidity, resulting in reduced crop production. A solution to water shortages is irrigation, which has made agriculture possible in many nonproductive areas (Kramer and Boyer, 1995). In the Wintergarden area of Texas, irrigation is also one of the major limiting factors in producing cotton and other crops.

Quantifying plant sensitivity to water deficit remains a challenge. A number of different quantification methods have been sought from the traditional measure of volumetric available soil water (Martin, 1940; Ritchie, 1981) to thermodynamic measures, which include fraction of extractable soil water (Ritchie, 1981; Sinclair, 2005), plant or soil water potential (Comstock and Mencuccini, 1998; Lamhamedi et al., 1992), relative plant tissue water content (Ritchie et al., 1990), canopy temperature (Idso et al., 1982; Jackson et al., 1981), and leaf- and whole-canopy gas exchanges (Faver et al., 1996; Marani et al., 1985; Baker et al., 1997). However, characteristic functions using the thermodynamic measures were not found to describe plant responses to either plant or soil water potential. Many studies now show that a two-segment model based on available soil water thoroughly describes the changes in plant water using daily plant gas exchange rate (Sadras and Milory, 1996).

More than 90% of the water for urban and agricultural use in the Wintergarden and Lower Rio Grande Valley comes either from the Rio Grande itself or the Edwards aquifer. As the Texas Legislature placed water restrictions on the farming industry by limiting growers to a maximum use of 6100 m^3 ha^{-1} of water per year in the Edwards Aquifer region, maximization of agricultural production

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efficiency has become a high priority for numerous studies in the Wintergarden area of Texas. Methods for improving water use efficiency described by some researchers (Taylor et al., 1983; Stewart and Nielsen, 1990) are (1) increasing the efficiency of water delivery and the timing of water application, (2) increasing the efficiency of water use by the plants, and (3) increasing the drought tolerance of the plants. The first method depends on mostly engineering and has been successful in improving productivity per unit of water delivered to the farm. The second and third methods depend on understanding physiological aspects and genetic characteristics of crops.

It is important to understand water requirement and physiological aspects of crops under limited irrigation management in order to achieve optimal production. The objectives of this research were to (1) investigate physiological responses of cotton based on leaf gas exchange measures under full and limited water availability using commercially available varieties at Uvalde, TX and (2) evaluate the feasibility to quantify plant sensitivity to water deficit with a measure of leaf canopy gas exchange. We also analyze factors affecting lint yield deduction.

2. Materials and methods

2.1. Experimental field and irrigation

Studies were performed at a Texas AgriLife Research field in Uvalde, Texas (29° 13′ 03″, 99° 45′ 26″; 283 m) in 2006 and 2007. The field (~4.8 ha) bedded in a circle was irrigated by a center pivot with a low energy precision application, LEPA, system. Soil type was an Uvalde silty clay soil (fine-silty, mixed, hyperthermic Aridic Calciustolls with a pH of 8.1). In 2006, six commercial cotton varieties from Bayer CropScience (Research Triangle Park, NC): ST5599, ST4892, ST4664, ST4700, ST5007, and 989B2R were planted at 20,647 seed ha^{-1} on 1 m row spacings on 11 April and harvested on 7 September. Likewise, four varieties from Bayer CropScience (RTP, NC) and Delta and Pine Land Company (Scott, MS): ST4554, DP555, DP164, and FM9063 were planted on 23 April and harvested on 17 October in 2007. The varieties were selected among those best adaptable to this region from commercially available varieties for both years. After having narrow yield variations among the varieties in 2006, varieties were selected considering more various genetic pools in 2007. The experiments in both years were arranged in a split-block design with each main plot (irrigation) replicated two times and each subplot (variety) replicated three times. A 90° wedge of the center pivot field was divided equally into 15° sections, which were maintained at 100%, 75%, and 50% crop evapotranspiration (ETc) values. The varieties were randomly arranged within each main plot.

Irrigation scheduling and ET regimes for the field were imposed according to calculations of the standardized ASCE_PM equation (ASCE-EWRI, 2005). Actual crop water use requirements for cotton were determined based on the relation to a well-watered reference grass. The equation was as follows:

$$\text{ETc} = K_c \times \text{ETo} \qquad (1)$$

where K_c is crop coefficient and ETo is reference evapotranspiration. We utilized the growth-stage-specific K_c values (Table 1), which were determined at the same study site (Piccinni et al., 2007). ET from a tall fescue grass (*Festuca arundinacea* Schreb.) with a height of 0.12 m and a surface resistance of 70 s m^{-1} was the ETo surface employed in K_c. The total amounts of irrigation from seeding to maturity (prior to defoliation) in 2006 and 2007 are presented with weather conditions in Table 2.

Table 1
Growth-stage-specific cotton crop coefficients (K_c) used.

Growth stage	Days after planting	K_c
Seeding	7	0.40
1st square	8–45	0.45
1st bloom	46–65	0.80
Max bloom	66–86	1.08
1st open	87–110	1.23
25% open	111–125	1.25
50% open	126–133	1.05
95% open	134–151	0.60
Pick	152–162	0.10

2.2. Data measurements and analysis

A neutron probe (530 DR Hydroprobe Probe Moisture Depth Gauge, Campbell Pacific Nuclear Corp. Int. Inc., Martinez, CA) was used to quantify soil moisture at various depths (20, 40, 60, 80, and 100 cm) during the crop growing season. Neutron probe data were obtained 13 times (8, 17, and 23 May; 1, 7, and 12 June; 20 and 28 July; and 4, 8, 10, 15, and 18 August) in 2006 and 10 times (6 and 22 June; 10 and 12 July; 2, 6, 10, 15, and 29 August; and 25 September) in 2007. After planting, neutron probe access tubes were installed at the center of each treatment plot. Volumetric water content, θ, was determined using a linear equation as follows:

$$\theta = a \times \text{CR} + b \qquad (2)$$

where a and b are coefficients and CR is the count ratio (count divided by standard count). The coefficients were determined for each soil depth by measuring soil moisture at different water contents with the neutron probe and by determining the gravimetric water content of soil samples. Fraction of transpirable soil water, FTSW, was calculated using the equation (Ritchie, 1981):

$$\text{FTSW} = \frac{\theta_a - \theta_{ll}}{\theta_{ul} - \theta_{ll}} \qquad (3)$$

where subscripts a, ul, and ll represent actual, lower limit, and upper limit of plant available water, respectively. The θ_{ll} and θ_{ul} used was 23.6% and 36.6%, respectively. The former was obtained from the NRCS soil survey (available at http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx), and the latter was determined using the method by Ratliff et al. (1983).

A LI-6400 (LI-COR, Lincoln, NE) with CO_2 injector and REDs (665 nm and 470 nm) light chamber were used to measure leaf gas exchange variables: leaf net assimilation, A_n (μmol (CO_2) m^{-2} s^{-1}); intracellular CO_2 concentration, C_i (μmol mol^{-1}); stomatal conductance, g (mol (H_2O) m^{-2} s^{-1}); transpiration rate, T_r (mmol (H_2O) m^{-2} s^{-1}); instantaneous water use efficiency, WUE$_i$; leaf temperature, T_L (°C); and vapor pressure deficit based on leaf temperature, VPD (kPa). Equations for calculating A_n, g, C_i, T_r, and

Table 2
Total irrigation applied and weather conditions during the cotton growing seasons in 2006 (11 April to 20 August) and 2007 (23 April to 10 September) in Uvalde, TX.

Year	Irrigation applied			Rainfall	Temperature	
	100% ETc	75% ETc	50% ETc		Max.	Min.
	mm				°C	
2006	487.7	382.3	291.6	71.4	35.0	21.3
2007	139.7	101.6	50.8	575.8	30.8	21.1
30 year[a]	–	–	–	315.2 (285.1)	34.5 (33.9)	20.7 (20.0)

[a] 30 year average (1971–2000): values in this row are seasonal averages from 23 April to 10 September while those in the parentheses are ones from 11 April to 20 August.

VPD are given in the LI-6400 user's manual (LI-COR Biosciences, 2002) after von Caemmerer and Farquhar (1981). WUE$_i$ was determined using the following equation:

$$WUE_i = \frac{A_n}{T_r} \tag{4}$$

Plant leaf level physiological responses were investigated at various photon flux densities (PFD) of photosynthetically active radiation (PAR 0 to 4000 umol m^{-2} s^{-1}) as well as various densities of CO_2 (0–800 umol mol^{-1}). We also measured leaf gas exchange with fixed cuvette conditions: a light intensity of 2000 μmol (photon) m^{-2} s^{-1} in both years; CO_2 concentrations of 200, 400, and 600 μmol mol^{-1} in 2006 and 400 μmol mol^{-1} in 2007. Measurements at the multiple CO_2 concentrations were not continued in 2007 because it was assumed that the multiple CO_2 impacts could be generally described by the measurements with the various CO_2 densities. This analysis was based on the measurements in 2006. While T_L was controlled at ~30 °C, humidity was controlled within a range 40–60% which was the best doable control condition on the photosynthesis system in the field. Measurements were made for uppermost fully expanded leaves on the plants every other week (30 May; 15 and 28 June; 10 and 25 July; 8 August) in 2006 and every week (12, 19, and 25 June; 2, 9, 16, and 27 July; and 2, 8, 14, and 25 August) in 2007 between 10:00 and 15:00 CDT on clear days.

Relative gas exchange, R_G, of A_n, g, and T_r was calculated using the following equation:

$$R_G = \frac{G_a - G_{ll}}{G_{ul} - G_{ll}} \tag{5}$$

where G_a represents the actual value of leaf gas exchange for each variable. G_{ul} and G_{ll} for g were defined as 1.5 and 0.5 μmol (H$_2$O) m^{-2} s^{-1}, based on a division by Flexas and Medrano (2002) and Medrano et al. (2002): $g > 1.5$, 'unstressed to mild drought'; $g < 0.5$, 'very severe drought'. Utilizing cotton physiological responses of leaf gas exchange (Ko et al., 2006, 2008), G_{ul} and G_{ll} for A_n and T_r were defined as follows: 40 and 25 μmol (CO$_2$) m^{-2} s^{-1} for A_n; 15 and 5 mmol (H$_2$O) m^{-2} s^{-1} for T_r; 35. RG of T_L and VPD was calculated using the following equation:

$$R_G = \frac{G_{ul} - G_a}{G_{ul} - G_{ll}} \tag{6}$$

G_{ul} and G_{ll} for T_L and VPD were defined as follows: 35 and 30 °C for T_L; 4.5 and 1.5 °C for VPD, based on cotton physiological responses of leaf gas exchange (Ko et al., 2006, 2008; Baker et al., 2007).

Five plant samples to quantify above-ground dry weight, AGDW (g m^{-2}), leaf area index, LAI (m^2 m^{-2}), and node numbers per plant were obtained on 21 August 2006 and 12 September 2007. A representative plant was randomly selected from each sample, and leaf area was measured with a LI-COR LI-3100 leaf area meter (LI-COR Inc., Lincoln, NE). The plants were weighed after being dried in a forced-air drying oven at 70 °C until the weight stabilized. Leaf area of the entire sample was calculated from the leaf area of the one plant and the ratio of the total dry weight of all plants divided by the dry weight of the one plant. Based on these techniques, we derived values for LAI and AGDW. Lint yields were determined by randomly sampling 3 m^2 from each plot. The measured errors (±1 SE) for the main plot were ~5–10, 7–15, and 2–7% of the means for AGDW, LAI, and lint yield, respectively, while those for the subplot were ~7–13, 8–17, and 3–5% of the means for AGDW, LAI, and lint yield, respectively.

The data were analyzed by analyses of variance using PROC GLM, standard errors of the mean using PROC MEANS, and Pearson's correlation coefficients using PROC COR (SAS version 9.1, Cary, NC). Treatment means were compared using the LSD and Duncan's Multiple Range tests at the 0.05 probability level.

Weather data were collected with a standard Campbell Scientific meteorological station (Campbell Scientific Inc., Logan, UT) at the Texas AgriLife Research and Extension Center (available at http://uvalde.tamu.edu/weather/weather.php).

3. Results and discussion

3.1. Soil moisture

Soil moisture values in most depths at most times during crop growing seasons in 2006 and 2007 were within the wilting point (23.6%) and field capacity (34.5%) of the Uvalde silty clay soil (http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx) except some cases at shallow soil depths (Figs. 1 and 2). The values in 2006 remained larger in May and smaller in August while the values in 2007 were comparatively largest in early August and smallest in late August. There were significant differences in soil moisture at most soil depths among irrigation treatments in 2006. On the other hand, there were no significant differences in soil moisture among irrigation treatments and within varieties at most soil depths in 2007. There were no interactions between the irrigation and variety treatments. The difference in soil moisture response between 2 years is attributable to differential irrigation that was more effectively applied in 2006 than in 2007. This was due to two extreme seasonal rainfalls, 71.4 mm in 2006 and 575.8 mm in 2007.
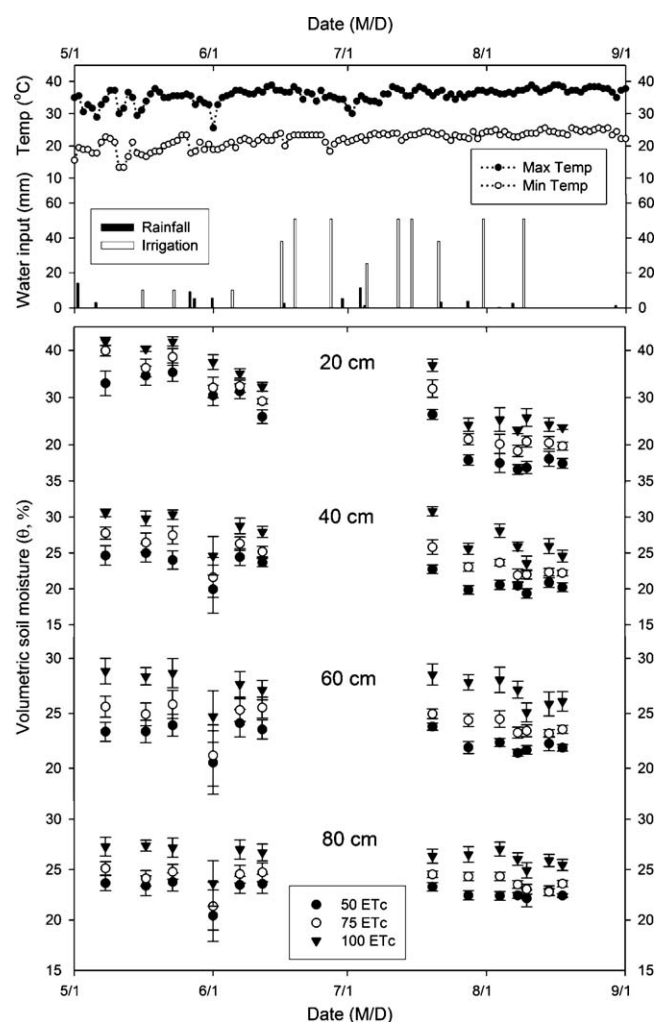

Fig. 1. Soil moisture at various depths as a function of different irrigation treatments (bottom), and irrigation and weather conditions (top) during the crop growing season in 2006. Vertical bars for soil moisture indicate ± 1 SE (n = 8).
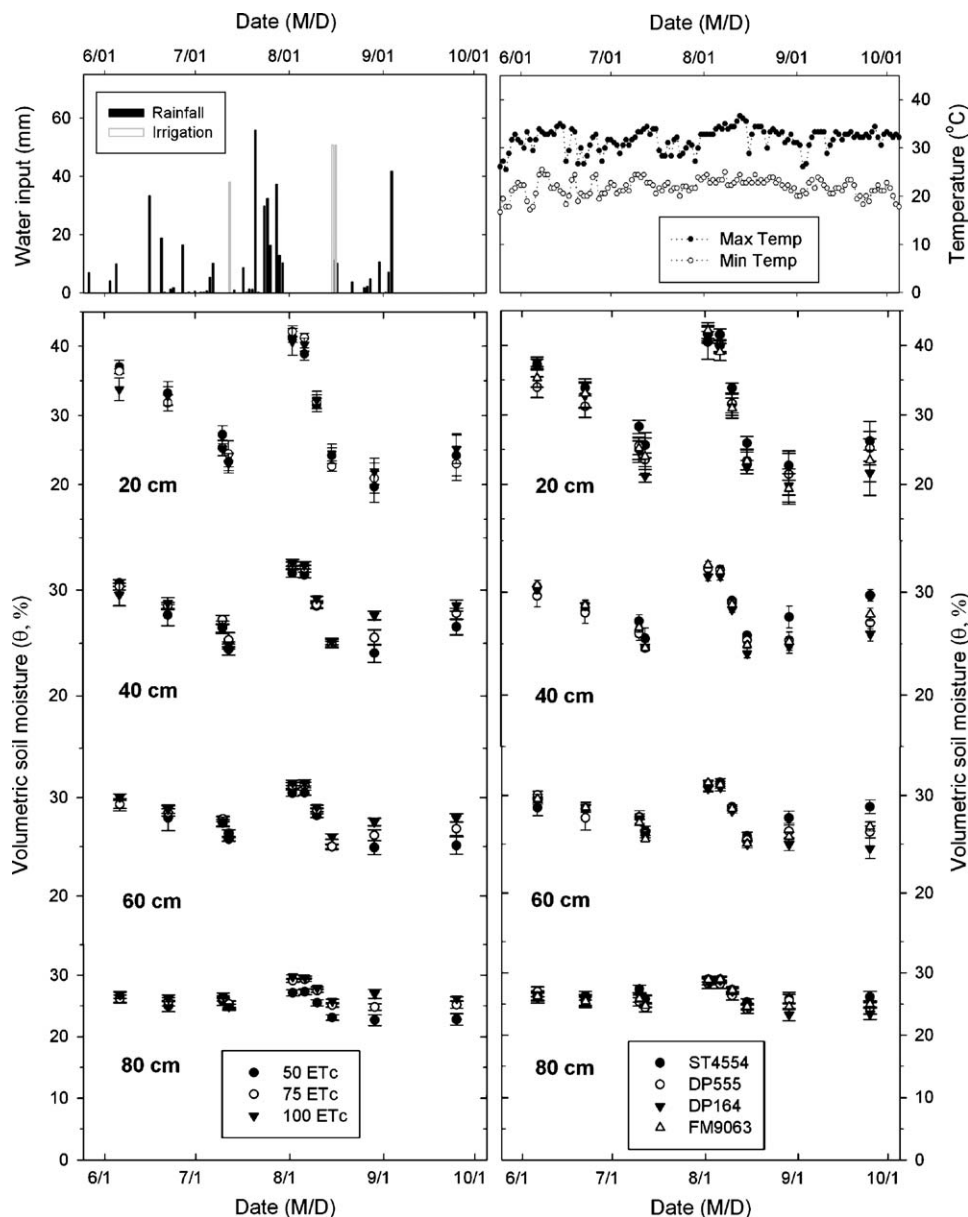
Fig. 2. Soil moisture at various depths as a function of different irrigation treatments and varieties (bottom), and irrigation and weather conditions (top) during the crop growing season in 2007. Vertical bars for soil moisture indicate ± 1 SE ($n = 12$ for irrigation and $n = 9$ for variety).

It has remained a challenge to answer to 'what is the independent variable in the plant-soil system that can be quantitatively and uniquely related to plant response to water deficits' (Sinclair, 2005). One of the answers is to use quantitative expressions of plant response in relation to volumetric soil moisture (Ritchie, 1981). Studies showed that a two segment model based on available soil water can be used to describe responses of the plant gas exchange variations to soil drying (Sadras and Milory, 1996; Sinclair, 2005). Assuming that soil moisture was reasonably monitored in this study, the soil moisture data are applied to quantify plant water status utilizing leaf gas exchange measurements in the following section (see Fig. 11).

3.2. Leaf gas exchange

The leaves of cotton plants under full irrigation were used to look into general leaf gas exchange responses to light intensity and CO_2. Leaf net assimilation (A_n) responded to photon flux density (PFD) with a threshold-like curve pattern, assumed being saturated

at ~2000 umol m^{-2} s^{-1} PFD (Fig. 3). Instantaneous water use efficiency (WUE$_i$) to PFD responded with a parabola-curve pattern, showing a peak at ~1500 umol m^{-2} s^{-1} PFD. A_n and WUE$_i$ to CO_2 responded with a threshold-like curve pattern, reaching a plateau at ~700 umol mol^{-1} CO_2 (Fig. 4). WUE$_i$ decreased with increased light intensity at 2000 umol m^{-2} s^{-1} of PFD while WUE$_i$ did not decrease with increased CO_2.

Crops sense and respond directly to rising light intensity and CO_2 concentration through leaf gas exchange (e.g., net assimilation and stomatal conductance). Our light response curve shows the classical pattern corresponding to the previous reports (Baker et al., 2007; Leaky et al., 2006). The present intercellular CO_2 response curve also shows the theoretical pattern described earlier (von Caemmerer, 2000; von Caemmerer and Farquhar, 1981). Elevated CO_2 increases net photosynthetic rate of plants until ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCo) is saturated with CO_2 (Ainsworth and Long, 2005; Ku and Edwards, 1977; Long et al., 2006; von Caemmerer and Farquhar, 1981). Elevated CO_2 also decreases stomatal aperture, which can reduce
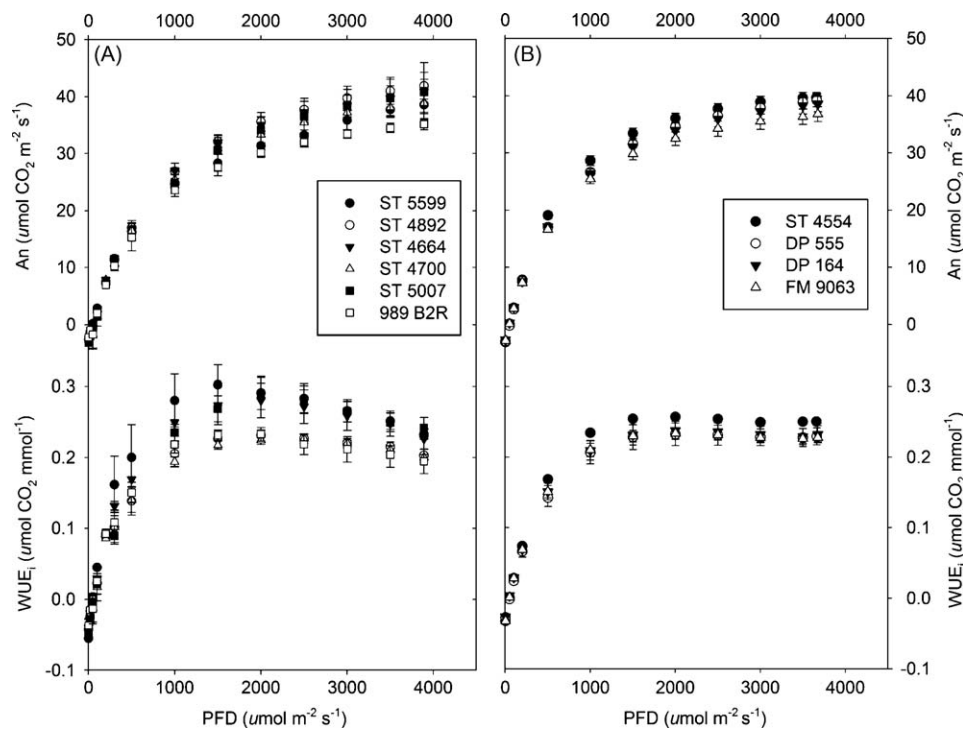
Fig. 3. Response curves of leaf net photosynthesis, A_n, and instantaneous water use efficiency, WUE_i, as a function of photon flux density (PFD) of photosynthetically active radiation for different cultivars in 2006 (A) and 2007 (B).

plant water use (Ainsworth and Long, 2005). Similarly, our result demonstrates that WUE_i increases with increased intercellular CO_2 concentration.

A_n and stomatal conductance (g) recorded peaks on 10 July and were comparatively small on 30 May during the season in 2006

(Fig. 5). While g values among irrigation treatments were significantly different after mid June, A_n values for 50% ETc were significantly different from those for 100 and 75% ETc after mid July (Fig. 5A and B). A_n and g values among different varieties varied during the season (Fig. 5C and D). A_n values increased at higher
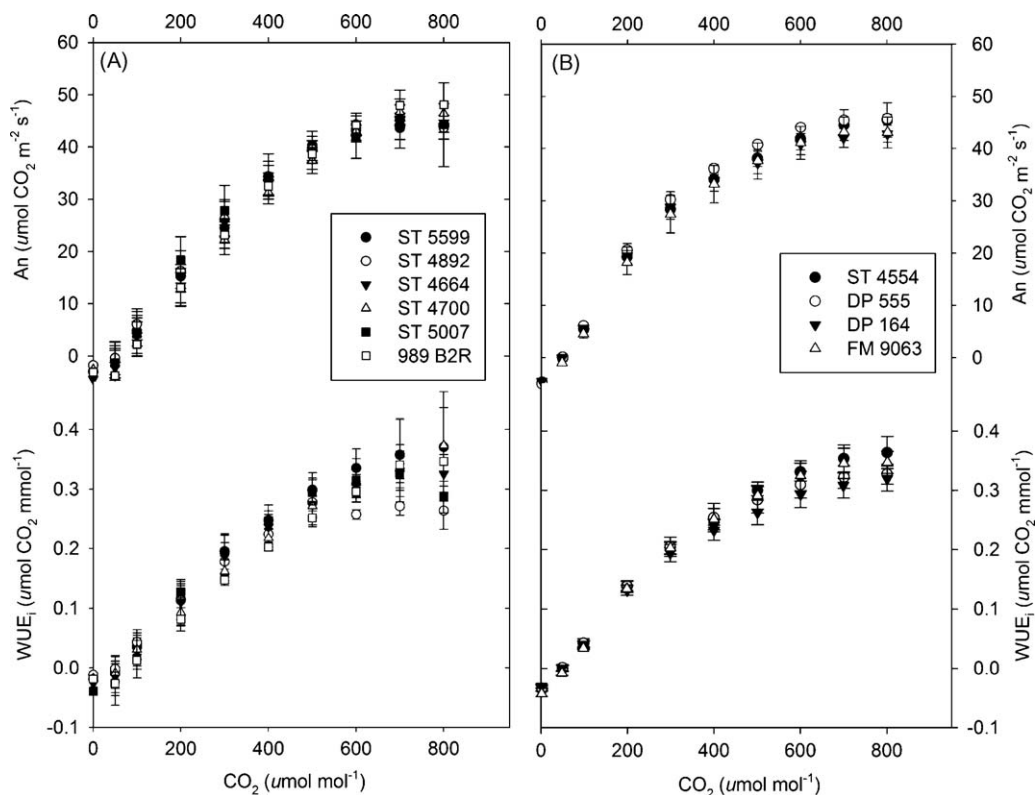

Fig. 4. Response curves of leaf net photosynthesis, A_n, and instantaneous water use efficiency, WUE_i, as a function of CO_2 concentration for different cultivars in 2006 (A) and 2007 (B).
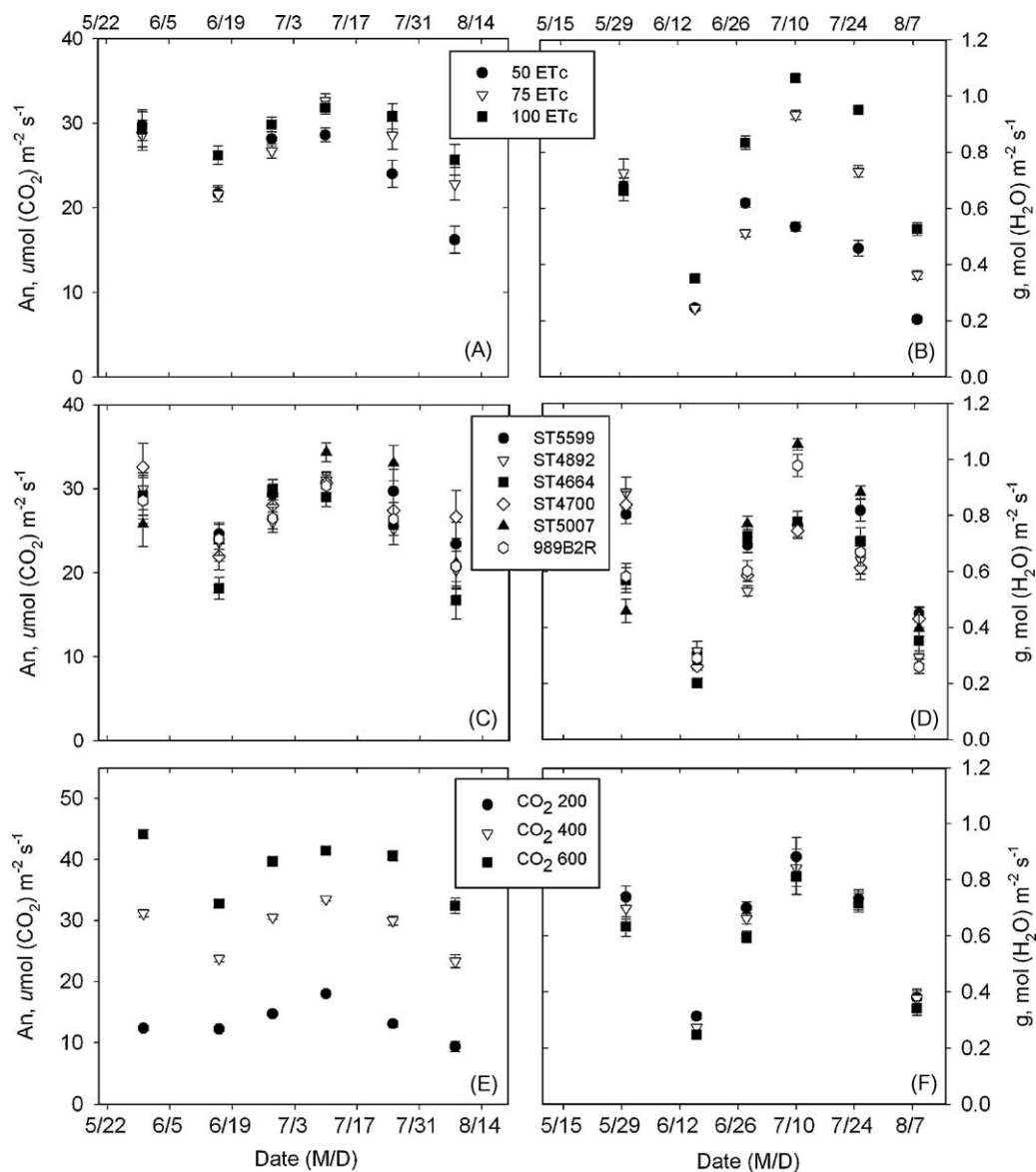
Fig. 5. Changes of leaf net assimilation, A_n, and stomatal conductance, g, for different irrigation treatments (A and B), varieties (C and D), and ambient CO_2 amounts (E and F) during the crop growing season in 2006. Vertical bars indicate ± 1 SE.

amounts of ambient CO_2 over the season while g values did not change or decreased (Fig. 5E and F). Transpiration rate (T_r) was highest on 10 July while WUE_i was highest on 15 June (Fig. 6). T_r values among the irrigation treatments were significantly different after mid June, while WUE_i values for 50% ETc were significantly different from those for 100 and 75% ETc in July (Fig. 6A and B). T_r and WUE_i values varied among the different varieties during the season (Fig. 6C and D). WUE_i values increased at higher amounts of ambient CO_2 over the season while T_r values did not (Fig. 6E and F). There were no interactions among the treatments for the variables at any time.

A_n increased with a linear phase until early July, in which A_n was highest, while g varied more among varieties and during the season in 2007 (Fig. 7). There were significant differences in A_n and g on 16 July within irrigation treatments while there were significant differences in late July and August within varieties. There were significant differences in T_r and WUE_i on 16 July and no significant differences were found on the other measured days during the season among the irrigation treatments (Fig. 8). Among varieties, T_r values for ST4554 were lower after mid July and no significant

differences in WUE_i were found on most measurement days. Likewise in 2006, there were no interactions among the treatments for the variables at any time.

Like for soil moisture, we assume that the difference in overall responses of leaf gas exchange between 2 years can be attributed to differential irrigation such that plants under the limited irrigations in 2006 experienced water stress. While there are a number of variables to monitor water deficit ranging from traditional measurement of volumetric available soil water to thermodynamic measurements, many studies demonstrate that plant gas exchange can provide a sensitive measure of the degree of crop drought stress (Baker et al., 2007; Faver et al., 1996; Flexas and Medrano, 2002; Medrano et al., 2002). The present results also demonstrate that water deficit can be qualitatively monitored using leaf gas exchange measurements as well. Perry et al. (1983) reported that there were apparent genetic differences in the ratios of photorespiration to net photosynthesis, remaining the possibility of genetically reducing photorespiration. Even though photorespiration is a vital component of the photosynthetic process in the species fixing CO_2 via C_3 pathway including cotton, it was not
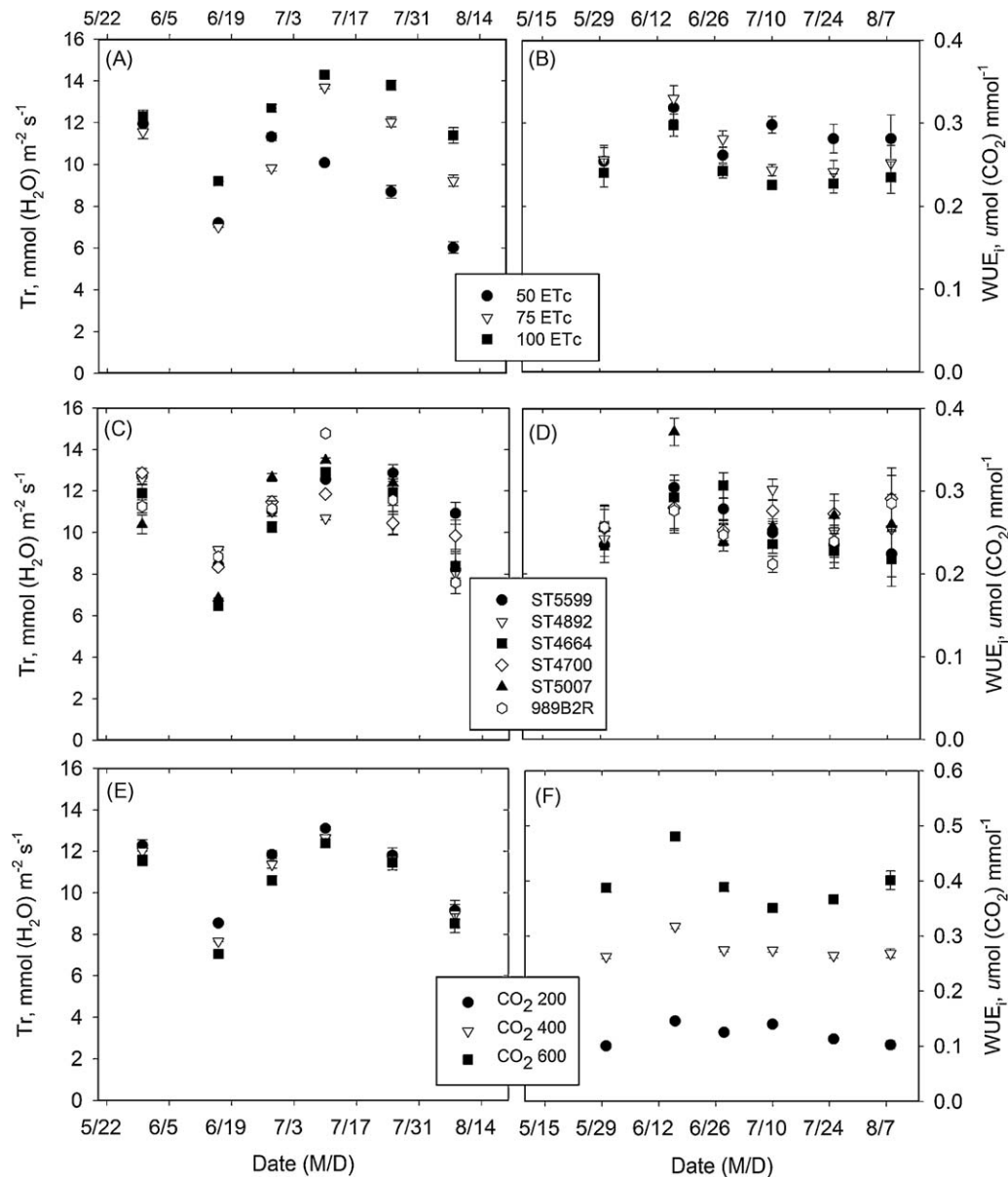
Fig. 6. Changes of transpiration rate, T_r, and instantaneous water use efficiency, WUE_i, for different irrigation treatments (A and B), varieties (C and D), and ambient CO_2 amounts (E and F) during the crop growing season in 2006. Vertical bars indicate ± 1 SE.

investigated in this study as we focused on monitoring plant water stress status using leaf gas exchange measurements. In addition, studies demonstrated that carbon isotope discrimination could be used to provide additional insight for photosynthetic metabolism in crops (Farquhar et al., 1989). We agree to the opinion by Cregg and Zhang (2000) that carbon isotope discrimination analysis has conceptual and practical advantages over measuring water use efficiency by instantaneous gas exchange measurements. Therefore, it is assumed that the carbon isotope discrimination methodology might provide better understanding of photosynthetic metabolism including water use efficiency for the current study.

Meanwhile, our study showed that A_n and WUE_i were increased by 30–35% and 30–40%, respectively, at 600 μmol (CO_2) m^{-2} s^{-1} in comparison to 400 μmol (CO_2) m^{-2} s^{-1}. The A_n response represents a theoretical A_n response of C_3 crops to elevated CO_2 from present-day 380 to 550 μmol (CO_2) m^{-2} s^{-1} (Long et al., 2006). In addition, the WUE_i response generally corresponds to an explanation by Ainsworth and Long (2005) that elevated CO_2 can reduce plant water use by decreasing stomatal aperture.

From leaf gas exchange data sets obtained in 2006 and 2007, the variables of A_n and g were plotted against other variables of interest to investigate relationships among them (Figs. 9 and 10). Again, our results present that A_n and WUE_i apparently increased at the higher CO_2 concentrations (e.g., 30–35% higher in A_n at 600 μmol (CO_2) m^{-2} s^{-1} than 400 μmol (CO_2) m^{-2} s^{-1}). The values of A_n increased until $\sim$40 μmol (CO_2) m^{-2} s^{-1} where C_i was 400 μmol mol^{-1}, making a plateau after that for the treatments of ambient CO_2 amounts of 400 and 600 μmol mol^{-1} (Fig. 9A). The values of A_n increased until $\sim$40 μmol (CO_2) m^{-2} s^{-1} as g and T_r increased (Fig. 9B and 9C). The values of A_n made a plateau at $\sim$0.15 μmol (CO_2) mmol^{-1} of WUE_i for the treatment of CO^2 200 and at $\sim$0.20 μmol (CO_2) mmol^{-1} of WUE_i for the treatments of CO^2 400 and 600 (Fig. 9D). The values of A_n generally decreased after 30 °C of leaf temperature (T_L) while A_n decreased from 1.5 kPa of VPD for the treatment of CO^2 200 and from $\sim$2.0 kPa of VPD for the treatments of CO^2 400 and 600 (Fig. 9E and F). The values of g increased depending on the values of C_i for each treatment of CO^2 200, 400, and 600 (Fig. 10A). The values of g increased as the values
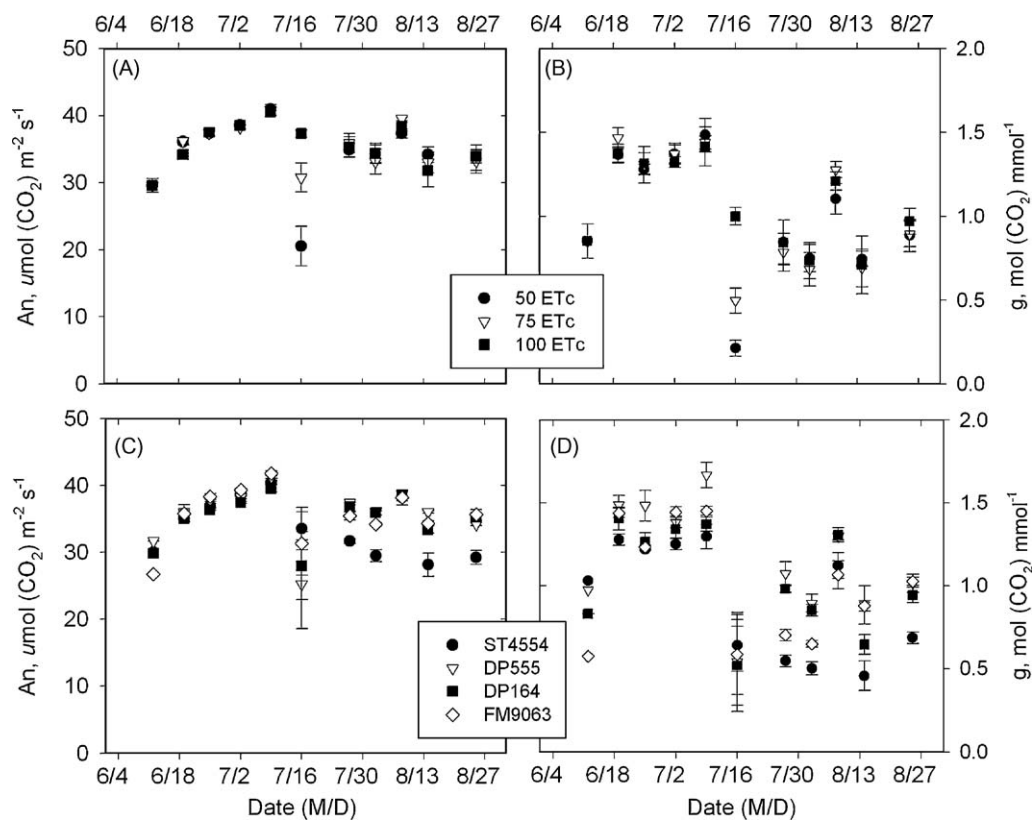
Fig. 7. Changes of leaf net assimilation, A_m, and stomatal conductance, g, for different irrigation treatments (A and B) and varieties (C and D) during the crop growing season in 2007. Vertical bars indicate ± 1 SE.

Fig. 8. Changes of transpiration rate, T_r, and instantaneous water use efficiency, WUE_i, for different irrigation treatments (A and B) and varieties (C and D) during the crop growing season in 2007. Vertical bars indicate ± 1 SE.
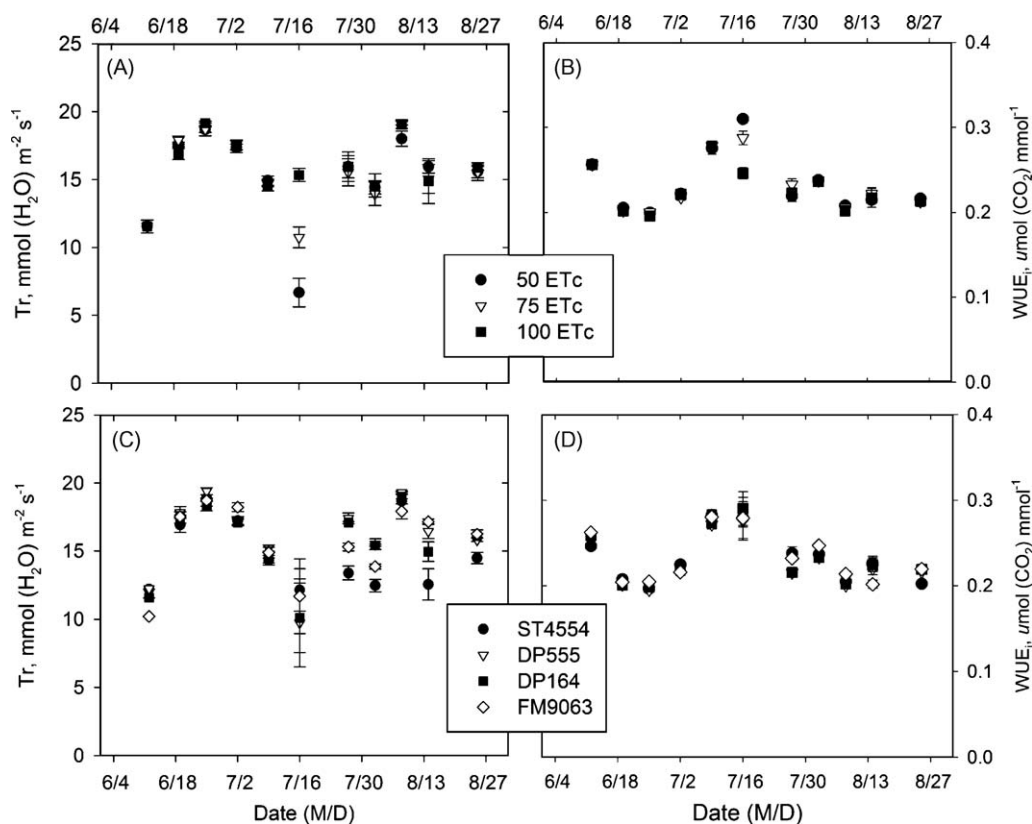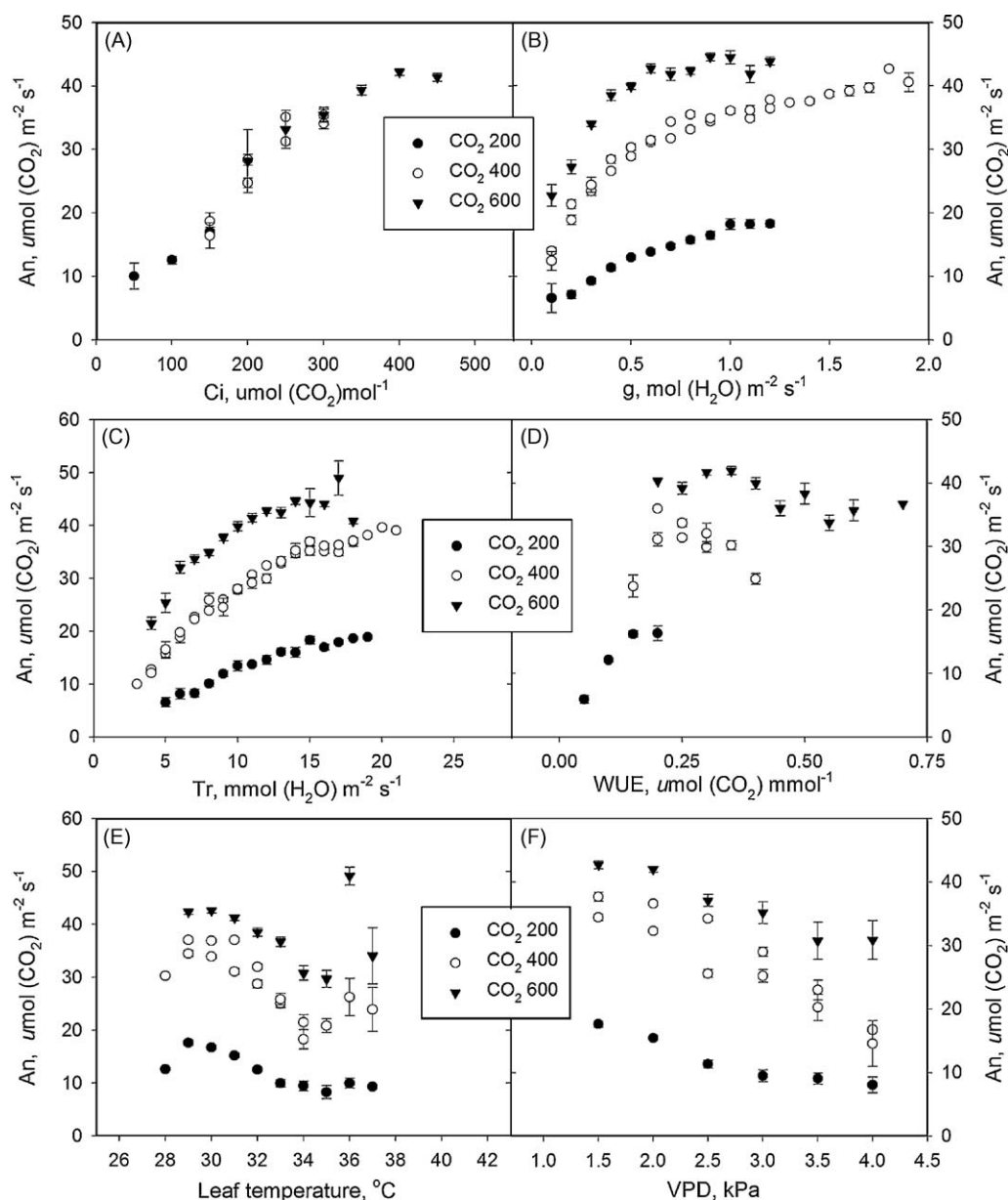
Fig. 9. Leaf net assimilation, A_n, in relation to intracellular CO_2 concentration, C_i, (A), stomatal conductance, g, (B), transpiration rate, T_r, (C), instantaneous water use efficiency, WUE_i, (D), leaf temperature (E), and vapor pressure deficit based on leaf temperature, VPD, (F) at ambient CO_2 densities of 200, 400, and 600 μmol mol^{-1} using data obtained in 2006 and 2007. Vertical bars indicate ± 1 SE.

of T_r increased (Fig. 10B). The values of g decreased after ~0.2 μmol (CO_2) mmol^{-1} of WUE_i, after 29 °C of leaf temperature, and after 1.5 kPa of VPD (Fig. 10C–E). We found that quadratic equations fit well for all relationships among the parameters shown for the treatment of CO^2 400. The equations are presented in Table 3 (A_n with C_i, g, T_r, WUE_i, T_L, and VPD) and in Table 4 (g with C_i, T_r, WUE_i, T_L, and VPD).

The relations among the leaf gas exchange variables reproduce the typical patterns on responses of A_n to g and C_i as well as those of g to C_i (Medrano et al., 2002; von Caemmerer, 2000; von Caemmerer and Farquhar, 1981). In addition, our results present that some parameters (e.g., T_r, T_L, and VPD) have strong correlations with A_n and g, which empirically measure plant physiological status. There have been many efforts to use plant canopy temperature (T_c) as well as the canopy minus air temperature differential (T_c–T_a) as a crop water stress index (Idso et al., 1981, 1982; Jackson et al., 1981; Wanjura et al., 1995; Wanjura and Upchurch, 2000). More recently, Baker et al. (2007) suggested using the term ($T_c - T_a$) either alone or

in combination with VPD as a predictor of water deficit status in cotton. Based on the results of the current study, we select the parameters A_n, g, T_r, T_L, and VPD to quantify plant water status as a function of soil moisture (see Fig. 11).

Table 3
Relationships between leaf net assimilation, A_n, and other variables (intracellular CO_2 concentration, C_i; stomatal conductance, g; transpiration rate, T_r; instantaneous water use efficiency, WUE_i; leaf temperature, T_L; and vapor pressure deficit based on leaf temperature, VPD) for the treatment of ambient CO^2 at 400 μmol mol^{-1}.

Equation[a]	R^2	RMSE
$-0.0007\ C_i^2 + 0.45\ C_i - 33.5$	0.95	1.91
$-10.3\ g^2 + 32.8\ g + 13.9$	0.94	1.94
$-0.09\ T_r^2 + 3.7\ T_r - 0.08$	0.99	0.99
$-495.6\ WUE_i^2 + 264.0\ WUE_i - 2.1$	0.64	2.69
$0.05\ T_L^2 - 5.3\ T_L + 142.4$	0.60	4.13
$-1.8\ VPD^2 + 1.9\ VPD + 37.5$	0.90	2.74

[a] $y = A_n$ in μmol (CO_2) m^{-2} s^{-1}; C_i in μmol mol^{-1}; g in mol (H_2O) m^{-2} s^{-1}; T_r in mmol (H_2O) m^{-2} s^{-1}; WUE_i in μmol (CO_2) mmol^{-1}; T_L in °C; VPD in kPa.
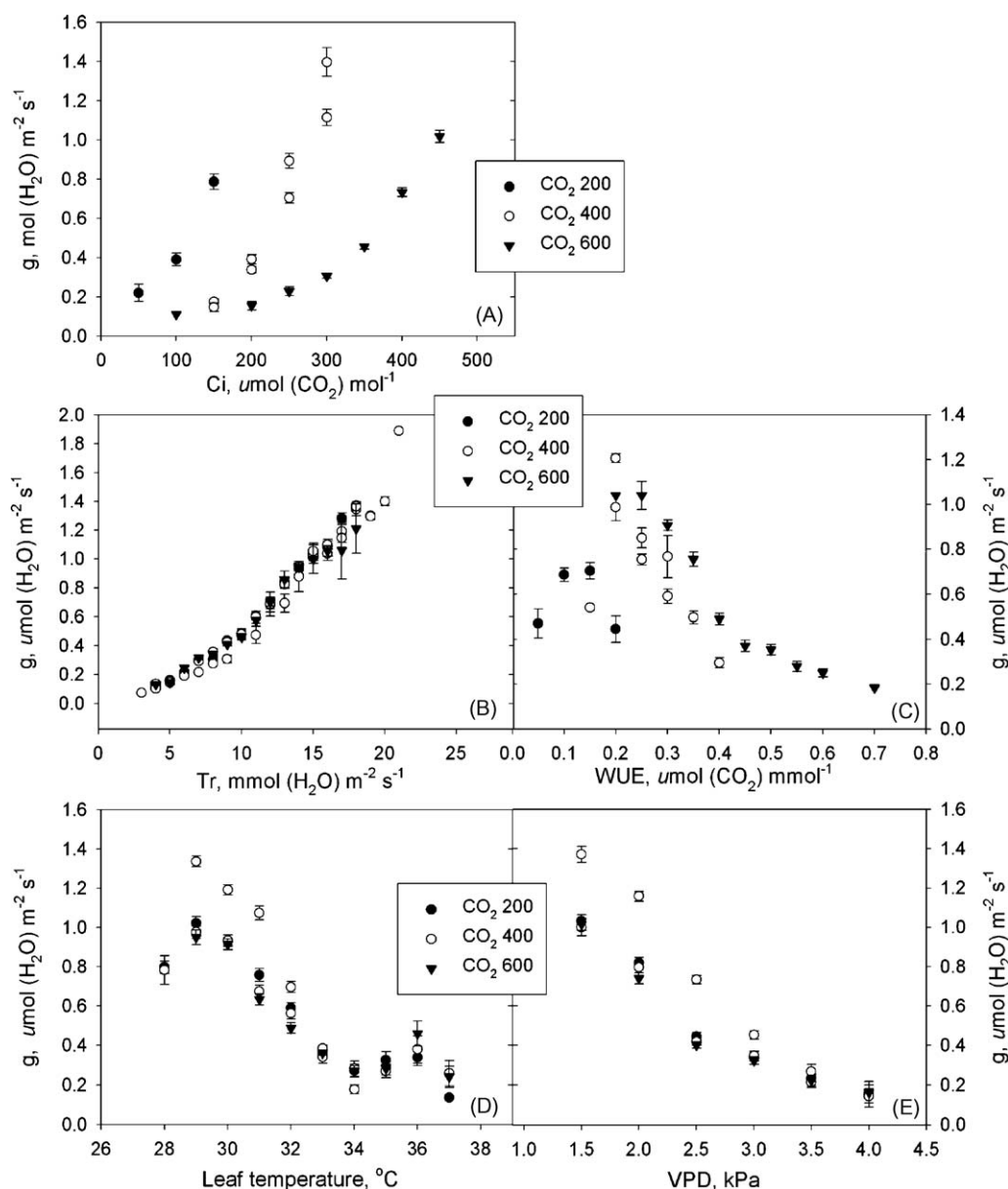
Fig. 10. Stomatal conductance, g, in relation to intracellular CO_2 concentration, C_i, (A), transpiration rate, T_r, (B), instantaneous water use efficiency, WUE_i, (C), leaf temperature (D), and vapor pressure deficit based on leaf temperature, VPD, (E) at ambient CO_2 densities of 200, 400, and 600 μmol mol^{-1} using data obtained in 2006 and 2007. Vertical bars indicate $\pm$ 1 SE.

3.3. Relationships among various parameters

Relationships were explored using Pearson's correlation coefficients among parameters of interest including lint yield,

plant growth variables, leaf gas exchange variables, and soil moisture at various depths (Table 5). Correlation coefficients of lint yield were comparatively larger with g, T_r, WUE_i, and soil moisture, θ, at 60 cm. Meanwhile, there were large correlation coefficients with each other among the other parameters of interest. In the relationships between the parameters with soil moisture at each depth, correlation coefficients were larger at 60 cm followed by 40 cm. When correlations between lint yield and the other parameters were analyzed in each month during the season (Table 6), large correlation coefficients were found in WUE_i and θ at 40, 60, and 80 cm in May; θ at 40, 60, and 80 cm in June; g, T_r, and θ at 40 and 60 cm in July; and A_n, g, and T_r in August.

It is assumed that lint yield was generally affected by θ at 40–60 cm over the growing seasons and gradually influenced by the above-ground plant physiological performances (i.e., g and T_r) as the seasons progressed. As plants develop in biomass, overall photosynthesis and growth respiration increase, responding to environmental stresses such as water deficit. This study presents

Table 4
Relationships between stomatal conductance, g, and other variables (intracellular CO_2 concentration, C_i; transpiration rate, T_r; instantaneous water use efficiency, WUE_i; leaf temperature, T_L; and vapor pressure deficit based on leaf temperature, VPD) for the treatment of ambient CO^2 at 400 μmol mol^{-1}.

Equation[a]	R^2	RMSE
$0.00003\, C_i^2 - 0.004\, C_i + 0.18$	0.96	0.11
$0.0003\, T_r^2 + 0.03\, T_r - 0.005$	0.98	0.07
$-21.5\, WUE_i^2 + 9.6\, WUE_i - 0.2$	0.62	0.19
$0.006\, T_L^2 - 0.5\, T_L + 10.3$	0.70	0.22
$0.11\, VPD^2 - 1.04\, VPD + 2.5$	0.89	0.15

[a] $y = g$ in mol (H_2O) m^{-2} s^{-1}; C_i in μmol mol^{-1}; T_r in mmol (H_2O) m^{-2} s^{-1}; WUE_i in μmol (CO_2) mmol^{-1}; T_L in °C; VPD in kPa.
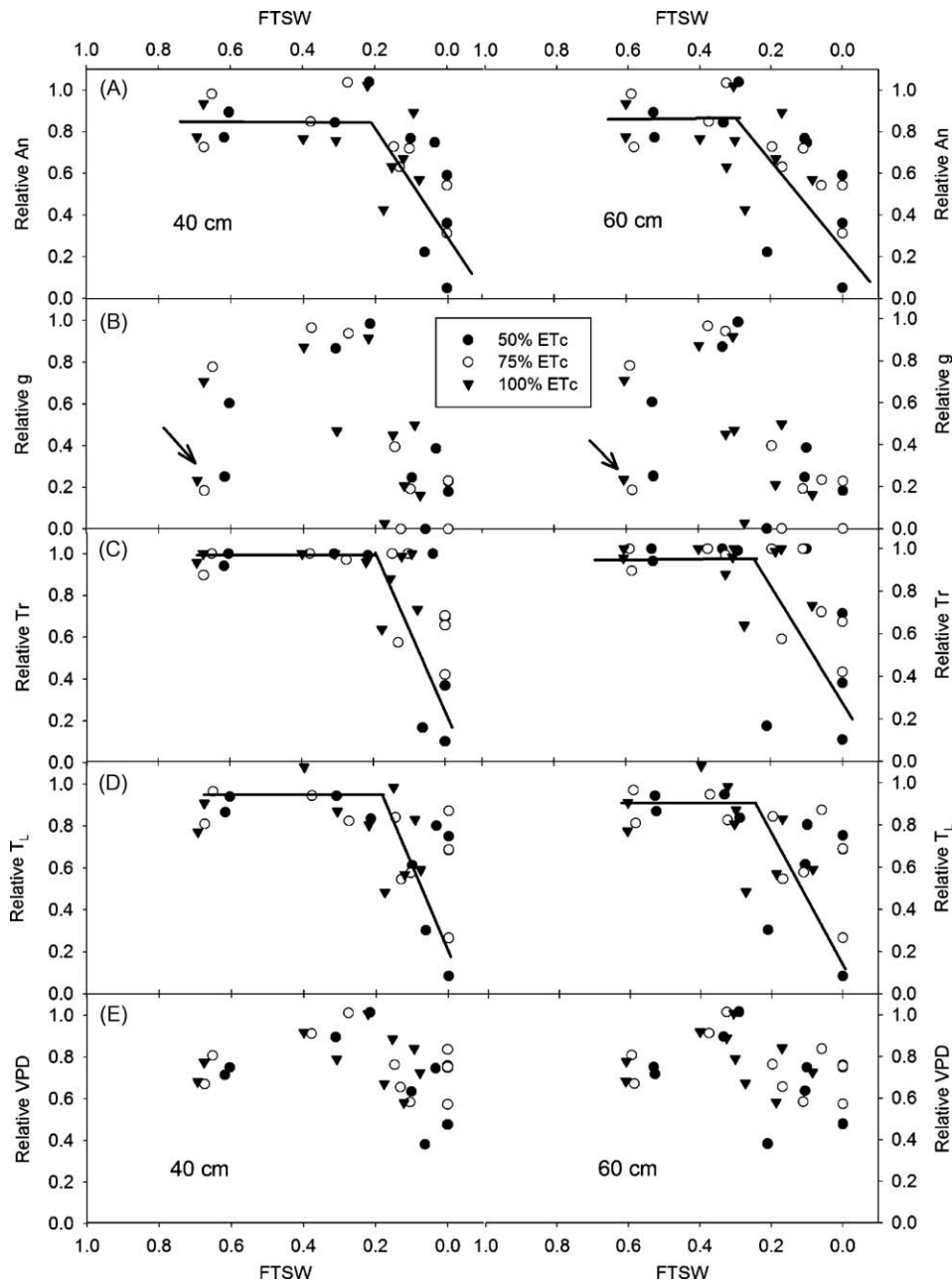
Fig. 11. Normalized leaf gas exchange rates in leaf net assimilation, A_n (A), stomatal conductance, g (B), and transpiration rate, T_r (C), leaf temperature, T_L (D) and vapour pressure deficit based on leaf temperature, VPD (E) plotted against fraction of transpirable soil water, FTSW, using data obtained in 2006 and 2007.

Table 5
Pearson's correlation coefficients among crop yield, above-ground dry weight (DW), leaf area index (LAI), node number, leaf net assimilation (A_n), stomatal conductance (g), transpiration rate (T_r), instantaneous water use efficiency (WUE_i), and soil moisture at various depths.

| | DW | LAI | Node | A_n | g | T_r | WUE_i | Soil moisture at each depth (cm) | | | | |
								20	40	60	80	100
Yield	0.38*	0.39*	0.48**	0.45*	0.57***	0.52**	−0.59***	0.46*	0.49*	0.51*	ns	ns
DW	–	0.91***	0.98***	0.96***	0.95***	0.95***	−0.84***	ns	0.70**	0.73***	0.59*	ns
LAI	0.91***	–	0.89***	0.91***	0.91***	0.89***	−0.77***	ns	0.72**	0.76***	0.67**	ns
Node	0.98***	0.89***	–	0.98***	0.98***	0.98***	−0.91***	0.51*	0.81***	0.84***	0.70**	ns
A_n	0.96***	0.91***	0.98***	–	0.98***	0.99***	−0.89***	0.62**	0.89***	0.90***	0.78***	ns
g	0.95***	0.91***	0.99***	0.98***	–	0.99***	−0.92***	0.62**	0.89***	0.90***	0.78***	ns
T_r	0.95***	0.89***	0.98***	0.99***	0.99***	–	−0.94***	0.63**	0.88***	0.90***	0.82***	ns
WUE_i	−0.84***	−0.77***	−0.91***	−0.89***	−0.94***	−0.94***	–	−0.81***	−0.98***	−0.99***	−0.90***	−0.44*

*, **, and *** represent significance at the 90%, 95%, and 99% probability levels, respectively; ns, not significant. Criteria for correlations (Cohen, 1988): 0.1–0.3: small; 0.3–0.5: medium; 0.5–1.0: large.

Table 6
Pearson's correlation coefficients between crop yield and other variables (leaf net assimilation, A_n; stomatal conductance, g; transpiration rate, T_r; instantaneous water use efficiency, WUE_i, and; soil moisture at various depths) in different months during the growing season.

	A_n	g	T_r	WUE_i	Soil moisture at each depth (cm)				
					20	40	60	80	100
May	ns	ns	ns	−0.68**	ns	0.95*	0.98*	0.99**	ns
June	0.46*	0.43*	0.43*	−0.42*	ns	0.51*	0.53*	0.61**	ns
July	0.37*	0.67***	0.56**	−0.49**	ns	0.53*	0.63**	ns	ns
August	0.50**	0.56**	0.52**	−0.45*	ns	0.45*	0.47*	ns	ns

*, **, and *** represent significance at the 90%, 95%, and 99% probability levels, respectively; ns, not significant. Criteria for correlations (Cohen, 1988): 0.1–0.3: small; 0.3–0.5: medium; 0.5–1.0: large.

that cotton lint yield had larger correlation coefficients with the parameters that respond to water deficit. While cotton yield is influenced by many factors genetically as well as environmentally, water deficit can be a major constraint of cotton yield (Kozlowski, 1972; Taylor et al., 1983; Yazar et al., 2002). Our results generally corresponded to this finding.

3.4. Transferable soil water vs. leaf gas exchange

As a method to quantify plant water deficit, we selected and normalized parameters of A_n, g, T_r, T_L, and VPD and plotted them against fraction of transferable soil water (FTSW) (Fig. 11). While quantifying plant water deficit was feasible using normalized A_n, T_r, and T_L, it was not feasible using normalized g and VPD. Relative A_n, T_r, and T_L started to decrease from FTSW 0.3 at 60 cm and FTSW 0.2 at 40 cm.

Previous studies demonstrated that leaf gas exchange could be a sensitive measure of drought stress with only leaf tissue expansion. It has generally been accepted that stomatal closure is the main determinant for decreased photosynthesis of plants experiencing mild to moderate drought stress (Charves, 1991; Cornic and Massacci, 1996; Ort et al., 1994; Sharkey, 1990). More recently, Flexas and Medrano (2002) and Medrano et al. (2002) divided g into four ranges based on the effect of g on various photosynthetic sub-processes from $g > 1.5\ \mu mol\ (H_2O)\ m^{-2}\ s^{-1}$, 'unstressed to mild drought' to $g < 0.5\ \mu mol\ (H_2O)\ m^{-2}\ s^{-1}$, 'very severe drought'. Our results on g did not correspond to their finding possibly due to the responses of g coupling with other physiological and environmental stress factors (e.g., arrows in Fig. 11). As water stress progresses, stomata close gradually and net assimilation decreases. Though soil water availability generally controls g, the performance of g is also affected by a complex interaction of factors internal and external to the plant leaf. Reduction in leaf gas exchange by reduction in g is attributable to the extent to which a plant is attached to its surrounding atmosphere (Jarvis and McNaughton, 1986). Meanwhile, some studies report nonstomatal effects of photosynthesis under severe water stress (e.g., Ephrath et al., 1993; Faver et al., 1996), i.e., inhibition or down-regulation of photosynthesis at the chloroplast level, resulting in reduced A_n at a given level of C_i. This was probably going on for some plant leaves under severe stress in the field condition of the current study but it could not be found out that our results on g are applicable to the nonstomatal effects.

As T_L and VPD showed relatively strong relations with A_n and g (see Figs. 9 and 10), we agree with the opinion by Baker et al. (2007) to use the combination of the term $(T_L − T_a)$ and VPD as a predictor of the degree of drought stress. Here our finding on quantitative relationships between FTSW and normalized leaf gas exchange rates also demonstrates that it is feasible to quantify plant water deficit using normalized A_n, T_r, and T_L. Especially, the result on T_r

appears to generally match with the result obtained with a theoretical approach (Sinclair, 2005).

4. Summary and conclusion

This study was conducted to evaluate physiological responses of cotton to various environmental conditions under limited water availability using commercially available varieties at Uvalde, TX. Our results demonstrated that plant water status under irrigation management can be qualitatively monitored using measures of soil moisture as well as leaf gas exchange. The results of leaf gas exchange measurement presented that some parameters (e.g., transpiration rate (T_r), leaf temperature (T_L), and vapor pressure deficit based on leaf temperature (VPD)) had relatively strong correlations with leaf net assimilation (A_n) and stomatal conductance (g). Lint yield showed strong correlations with g, T_r, instantaneous water use efficiency (WUE_i), and soil moisture at 60 cm depth. We also showed that A_n and WUE_i were apparently increased at the higher CO_2 concentrations (e.g., 30–35% and 30–40% higher in A_n and WUE_i, respectively, at 600 $\mu mol\ (CO_2)$ $m^{-2}\ s^{-1}$ than 400 $\mu mol\ (CO_2)\ m^{-2}\ s^{-1}$). We assume that the measures of the leaf gas exchange parameters can be useful to qualitatively describe yield reduction due to water deficit. A method to quantify plant water deficit was presented using A_n, g, T_r, and T_L as parameters, and quantifying plant water deficit appears to be feasible using normalized A_n, T_L, and T_r.

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